

**ENTEROVIRUS D68 (EV-D68)
SPECIMEN COLLECTION/SUBMISSION INSTRUCTIONS
September 2014**

DCLS will provide the collection materials and testing services in support of outbreak investigations of EV-D68 infections. Kits may be obtained from the Sample Kit Office at (804) 648-4480 x104 for patients meeting case definition criteria and after approval by the Virginia Department of Health. Each kit provides enough material to sample five patients and is identical to DCLS' influenza and virus isolation test kits. Collect the sample(s) as close to clinical onset as possible.

Kit Contents:

- 5 - 8x10 biohazard bag with pouch & absorbent pad
- 1 - Secondary containment vessel (black and white)
- 1 - Instructions for the submission of specimens for influenza and other viruses
- 2 - Ice packs (additional supplies upon request)
- 1 - Insulated shipper per IATA
- 1 - Itemized list of contents card
- 5 - Clinical microbiology/virology request form
- 5 - Sterile (Dacron) polyester-tipped applicator swab
- 5 - Sterile viral transport media (VTM)
- Sterile urine cups available upon request

Appropriate Sample Types in order of priority:

1. Upper Respiratory Tract Specimen	Nasopharyngeal Swab (NP)
AND	
2. Stool	Formed or liquid

* Note: NP swabs may be used for additional testing to rule-out other viral respiratory pathogens.

Testing Performed and Associated Turnaround Times:

- Viral culture and identification can be used to grow viruses in the Enterovirus genus and to screen for their presence using fluorescent antibody (FA) testing using NP and stool samples.
- Polymerase Chain Reaction (PCR) can detect the presence/absence of common respiratory viruses (Influenza A/H1, Influenza A/H3, Influenza A 2009 H1N1, Influenza B, Rhinovirus, RSV, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Human Metapneumovirus, and Adenovirus B/C/E) in NP samples only.
- For high probability cases, an additional PCR assay can be employed to presumptively detect the presence of Enterovirus in NP samples only.

Important: At the present time, viral culture, FA testing and PCR cannot definitively identify EV-D68. The information provided by these testing methods can only presumptively identify the presence of Enterovirus. Samples that are presumptively positive for Enterovirus or outbreaks samples where an etiologic agent cannot be identified will be forwarded to CDC for further testing.

- Turnaround Time (TAT) is highly dependent upon sample collection, storage, and handling.
 - Viral culture and identification TAT: 48 hour – 14 days
 - PCR TAT: 24-48 hours

Collection Instructions for NP Swab Specimens:

- Instruct the patient to sit with head tilted back slightly. Gently push the tip of the patient's nose back with your thumb.
- Insert the NP swab into the nose and back to the nasopharynx. The patient's eyes will momentarily tear. Slowly rotate the swab as it is being withdrawn.
- Repeat the process using the same swab in the second nostril.
- Place the swab into VTM and label it with the patient's name and date of collection.

- Promptly refrigerate the sample until it can be shipped to the laboratory.
- DCLS must receive the sample within **72 hours of collection** using the provided shipper and cold packs.

Collection Instructions for Stool

- Stool may be formed or liquid.
- Place collected stool into a sterile collection cup.
- Label the sterile cup with the patient's name and date of collection.
- Promptly refrigerate the sample until it can be shipped to the laboratory.
- DCLS must receive the sample within **72 hours of collection** using the provided shipper and cold packs.

Instructions for Specimen Transport

The DCLS courier can be used to ship all samples related to EV-D68. Use the instructions below to package and ship the specimens via courier or FEDEX.

All samples should be shipped refrigerated. Package specimen for transport to the laboratory in compliance with shipping regulations detailed in IATA 1.5 AND 49 CFR Section 1720700 [U.S. Department of Transportation] using the provided shipper.

1. Ensure that specimen is properly labeled and the Green Clinical Microbiology/Virology Request Form is complete (Include outbreak ID number on the request form). The patient identification on the form and collection container should be identical.
2. Place the samples into the 95 kPa secondary containment bags.
3. Place the secondary containment bags into the shipper
4. Place frozen cold packs into the shipper. Additional cold packs and newspaper may be used, if available, to better control the temperature.
5. Insert the completed Green Clinical Microbiology/Virology Request form into the outer pocket of the Ziploc bag and place into the insulated shipper.
6. Place the styrofoam lid on top of the box to seal its contents.
7. Securely seal shipper, following the closing instructions found on the shipper.
8. If package will be delivered by FEDEX, then proceed to step #10.
9. If your package is being delivered to the laboratory by any method other than FEDEX, proceed to step 15.
10. Place the "UN3373 Biological Substance Category B" label on a side of the box not occupied by the directional labels.
11. Complete Section 1 of the FedEx billable stamp with your name (preferably, or facility name), address, and phone number.
12. The left side of the FedEx billable stamp is for your records and the right side of the stamp should be placed on the top of the package.
13. Peel off the backing of the FedEx billable stamp and affix to the outside of the cardboard shipping box. This stamp should not cover any labeling and should not extend beyond any edge of the package.
14. **Call FedEx for a pick up at 1-800-463-3339 or contact your Health Department Epidemiology representative regarding package drop off for delivery to the lab. Do not ship on Friday or before a holiday.**
15. **Ship specimens without delay. Specimens must be received at DCLS within 72 hours of collection.**

Result Reporting: Results will be telephoned to the Virginia Department of Health and the submitter.

Additional Information: Please contact Dr. Marilyn Bibbs Freeman (x270), Sean Kelly (x227) or Dr. Angela Fritzinger (x205), for questions regarding sample collection, sample handling, or laboratory testing.